EXHIBIT 1

ANDREW GOSEA:

RECEIVED 100 cc CORD BLOOD (4) CPD)
PBE mono 30%
Pelys 70%

added 15 ml 1xPBS to bring to incubation volume of 45 ml.
Added 1.5 ml antibody (12.8)
incubated 25 ml.
Primed CellPro "cepvate".

Spun cells. Resuspended in 1x PBS to a final volume of 300cc in bag.

Ran through column.

Unadsorbed portion → spun down. and consolidated in 1xPBS for incubation.

75ml for incubation (added HSA)
1.5ml antibody (12.8) 20min. Spun down following incubation. Rsp'd to Vol. of 300cc in bag
Ran through 2nd column.

Stem cell portions from Runs 1 & 2 were combined (after ~~day~~ counts done and samples removed for staining)

Total cells 2.8x10^9 for transduction

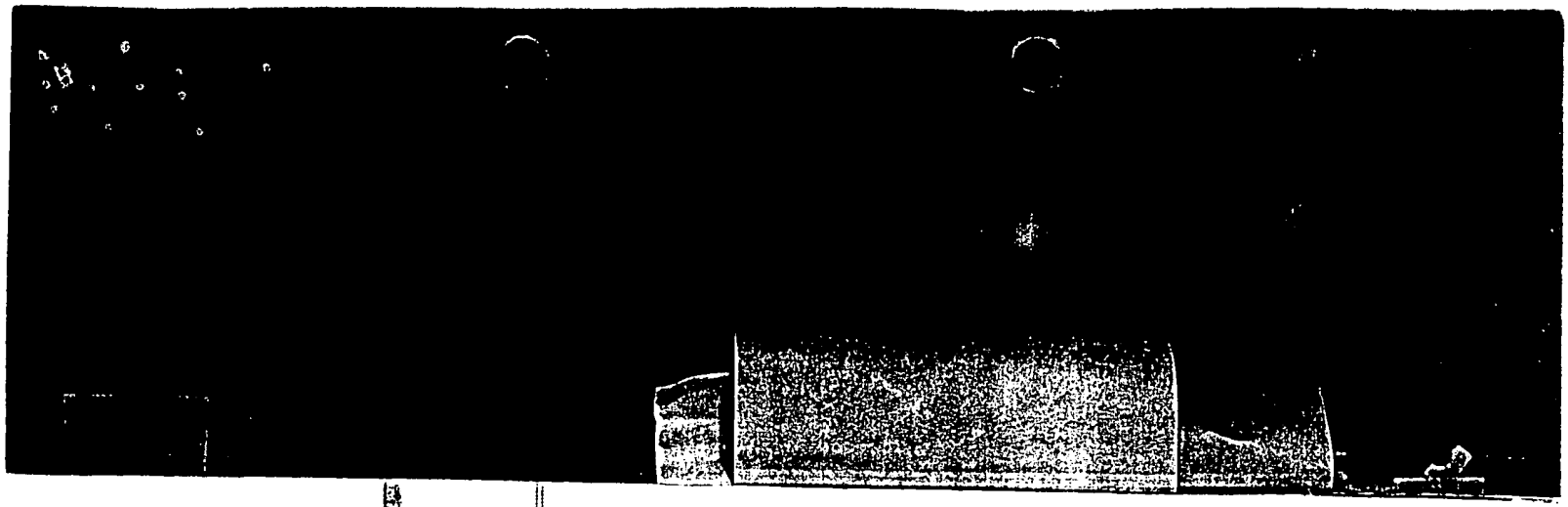
BBmm +

1L3 SAND
wan
1L6 SAND
wan
SLF ANGE
wan

final concer
will be dil
Cells are
concentra

BBmm: PB
PBS

2.8x10^9 cells
Put in



BBMM + 316/SCF (for 500ml of media)

IL3 SANDOZ # Y0230892 stock at 150ug/ml
want final: 20ng/ml x 2 ∴ 20ug 133ul add

IL6 SANDOZ # Y0150392 stock at 150ug/ml
want final: 50ng/ml x 2 ∴ 50ug 333ul add

SCF AMGEN # 15092 stock at 1.5mg/ml = 1500ug/ml
want final: 100ng/ml x 2 ∴ 100ug 67ul add

final concentrations are doubled since the media will be diluted 1:2 w/ viral supernatant. Cells are therefore incubated with the correct concentrations.

BBMM: FBS Genuni LOT# AC0023H
BSA #115

2.8x10⁶ cells want final: 5x10⁶ cells.
Put in 2 T-75 30ml each: 15ml B365 051193
15ml LASN GT LOT# 53
+ protamine sulfate 240ul of 1:20 diluted stock

D)

xubation

35 to bag.

7.

pan down of 300c in bag.

1 & 2 toge and

Cord Blood cells pre processing:

CFUs: SCT 143

	Plate #	Sample	# Cells	# ul/ml media
-G418	1ab	Gdoea UCB	5×10^4	50
+G418	2ab	↓	↓	50
-G418	3ab		1×10^5	100
+G418	4ab	↓	↓	100

Start:

absorbed fraction:

absorbed fraction

CFUs Post transduction: SCT 144

	plate #	# CELLS	# ul
ØG418	1ab	500	7
↓	2ab	1000	14
	3ab	2000	28
+G418	4ab	500	7
↓	5ab	1000	14
	6ab	2000	28

(yields) absorbed

↑ count:

$\bar{x} = 34$

$\times 2 \times 10^4 = 6.8 \times 10^5$ C/ml

$\times 5.5$ ml $= 3.7 \times 10^6$ C

Infused on 5/15/93

Non-transduced stem cells

absorbed

G418			
Ø	7ab	1000	20
+	8ab	2000	40

Start:

	Pre	Post ab
5×10^8 C	0.71%	0.22%

*ul/ml media

*3H+ = 3.6×10^6 C = 1.1×10^6 C

30
30
100
100

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absorbed fraction #1:

	FL1 FL2 gate	FL1 FSC gate
2×10^6 C	31.94%	20.81%
*3H+ =	0.64×10^6 C	0.42×10^6 C

absorbed fraction #2:

0.8×10^6 C	2.46%	5.80%
*3H+c =	0.02×10^6 C	0.05×10^6 C

set 144

(yields)

absorbed #1:

Pre & FL1/FL2 gate: $\frac{0.64 \times 10^6 C}{3.6 \times 10^6 C} =$ 17.8%

Pre & FL1/FSC gate: $\frac{0.42 \times 10^6 C}{3.6 \times 10^6 C} =$ 11.7%

Post ab & FL1/FL2 gate: $\frac{0.64 \times 10^6 C}{1.1 \times 10^6 C} =$ 58.2%

Post ab & FL1/FSC gate: $\frac{0.42 \times 10^6 C}{1.1 \times 10^6 C} =$ 38.2%

absorbed #2: Pre & FL1/FL2 | Pre & FL1/FSC

[illegible]

ZACHARY RIGGINS: 5/14/93

RECD 200cc COLD BLOOD

PRE: mono 109 / poly 109

218 x 50 = 10.9 x 10^6 c/ml
x 200ml = 2.2 x 10^9 c start

Added 3 vials (4.5ml) 12.8 ab
inc. 25 min.

Spun down. Rspd in 1xPBS to 300ml in bag.

Ran through column:

Spun down unabsorbed fraction for 2nd ab incubation.

Spun stem cell fraction to Rspd in smaller volume for count.

Counts:

unabsorbed		stem	
mono	poly	mono	poly
67	102	172	16

169 x 30 x 10^3
= 8.5 x 10^6 c/ml x 225ml
= 1.9 x 10^9 c

188 x 2 x 10^4
= 3.8 x 10^6 c/ml x 5.5ml
= 20.7 x 10^6 c

incubated unadsorbed fraction w/ 4.5ml
12.8 ab. for 25 min
Spun down.
Put in 300ml in bag (w/ 1xPBS)
Ran through 2nd column.

Counts:

unadsorbed		stem	
mono	poly	mono	poly
30	33	58	4

$63 \times 5 \times 10^3$
3.15×10^6 C/ml x 600ml
$= 1.9 \times 10^9$ C

$62 \times 2 \times 10^4$
$= 1.2 \times 10^6$ C/ml
x 5ml = 1.6×10^6 C

⇓

percolled/ficolled
free ⇒ LUT(2)

combined stem cell fractions

26×10^6 C for transduction
want final [] = 5×10^4 C/ml — 520ml total
÷2 = 260ml supe
260ml media

13 flasks 40ml/flask — 20ml supe
20ml media (B365)
+ 320ul protamine sulfate
1:20

LASN supe 539 (bottles 18/19)

CFUs:

5CT145

PRE

Plate #	Sample	# cells	# ml
1ab	PRE trans (-G418)	5×10^4	5
2ab	↓ (+G418)	↓	5

BBMM + 3116/SCF:

1L3 Sandoz # yo230292

1L6 Sandoz # yo150392

SCF AMGEN # 150952

Took sample to micro for sterility ✓
each day of transduction pd. 0
Stat Gram stain done (negative)
before cells were given to baby.

5/15/93 4pm 2nd transduction:
Spun cells down from each flask
Resp in fresh media & LASN supe
added Protamine sulfate

5/16/93 3rd transduction 330pm.
Repeated above.

5/17

1

CAX

PLT

REM

POST

DG

↓

+G4

↓

DG418

+G4

5/17/93 cells washed 4x:
3x in 1xPBS + P/S
last wash in RPMI (no P/S)

count: $\bar{x} = 15 \times 10 \times 10^4 = 15 \times 10^5$ c
$\times 40\,ml = 600 \times 10^6$ c

Put in 5cc into 10cc syringe

Reinfused on 5/17/93. (UCSF)

Post trans. CFUs: 5a/4b

sample plate #		# cells	# µl
DG418	1ab	500	4
	2ab	1000	8
↓	3ab	2000	16
+G418	4ab	500	4
	5ab	1000	8
↓	6ab	2000	16
DG418	7ab	1000	24
+G418	8ab	1000	24

µl

5
5

Hy ✓
O
wire)
o baby
tion:
n flask
FSN supe

330pm